BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

REMARKS

Claims 2-9, not 1-9 as stated in the Office Action Summary, are pending in the application. Upon entry of the amendment herein, claims 2-9 remain pending in the application; claim 2 has been amended.

Claim 2, and claims 3-9 dependent therefrom, remain rejected as indefinite "for reason of record." The Examiner maintains that claim 2 omits essential steps; in particular, it is asserted that "a correlation step correlating the 'light energy measured' with the 'peptidoglycan synthesis' is missing and/or not distinctly recited in the rejected claim." In the April 17, 2002 discussion with Applicants' agent, the Examiner confirmed that page 6, lines 16-24 of the instant specification, cited in Applicants' previous response, provides support for the alleged omitted essential steps. The Examiner also affirmed her belief that appropriate language from that specification passage must be inserted into claim 2.

In the interest of expediting prosecution of the application, then, claim 2 has been further amended to recite elements of the defining disclosure cited by Applicants. Language from page 6, lines 9 and 10 has also been incorporated into the claim. The inserted elements are along the lines of those discussed, and agreed upon, by the Examiner and Applicants' agent Furthermore, the amendment of claim 2 meets

all of the criteria for entry of amendments after final rejection; entry of the amendment is respectfully requested. In light of the amendment, the indefiniteness rejection should be withdrawn, and such withdrawal is respectfully requested.

Applicants acknowledge the Examiner's (tacit) withdrawal of all other previous indefiniteness rejections of the claims.

The rejection of the claims under 35 USC §103(a) as being obvious over Elhammer in view of Mengin-Lecreulx et al. and Kohlrausch et al. has been maintained, again "for reason of record." The Examiner maintains that the Elhammer disclosure of the application of SPA in studying cellular processes combined with the alleged teachings of Mengin-Lecreulx and Kohlrausch with regard to peptidoglycan synthesis in *E. coli* would have led one of ordinary skill in the art to the instant invention. Again, Applicants emphatically disagree with the Examiner's assessment.

The Examiner states that "[A]bsent unexpected results, it would have been obvious to have applied" SPA "in detecting peptidoglycan synthesis." However, unexpected results are not the appropriate standard in the present context. The Examiner appears to believe that mere knowledge of the pathway of peptidoglycan synthesis, provided by the secondary references, would have enabled one of skill in the art to fill in the gaps in the teaching of the primary reference, and would somehow have

countered some of the discouraging prior art teachings, thus motivating the skilled artisan to arrive at the instant invention. However, such knowledge provides no guidance for overcoming the obstacles well known in the art at that time, nor does it provide any encouragement to try to overcome said obstacles.

It must be emphasized that the Examiner's assessment of the "obviousness" of the instant invention could only have been arrived at by the use of impermissible hindsight. It must be appreciated that the enzymes used in the assay according to the instant invention are those involved in the final stages of peptidoglycan synthesis (classically referred to as stage 2 and 3 peptidoglycan synthesis where peptidoglycan is the final product) and represent so-called "downstream enzymes." As disclosed on page 3, lines 6-25 of the present application, methods for assaying the (downstream) enzymes have typically relied on paper chromatography, and this is confirmed, for example, in the Mengin-Lecreulx reference (see the experimental section on page 4628, left-hand column.) However, a drawback of using paper chromatography is that it is difficult to control the reaction conditions and, furthermore, it is entirely unsuitable for high throughput screening of compounds.

That the assay of downstream enzymes is recognized in the art to be difficult is illustrated, for example, in a passage

from the first paragraph of the article of Men, et al., J. Am. Chem. Soc. **1998**, *120*, 2484-2485 (copy enclosed), published only a few months before the earliest claimed priority date of the present application:

> Although remarkable progress has been made in characterizing some of the early enzymes in the biosynthetic pathway, the downstream enzymes have proven exceedingly difficult to study. This is partly because the downstream enzymes are membrane-associated, making them intrinsically hard to handle, and partly because substrates for many of the enzymes are not readily available. These problems have impeded the development of active assays suitable for detailed mechanistic investigations of the downstream enzymes.

Bearing in mind that scintillation proximity assay (SPA) technology has been known from U.S. Patent No. 4,568,649 since 1986 (see page 6, lines 4 and 5 of Elhammer), the Examiner's contention that the assay according to the present invention is obvious is simply not credible. If such were the case, it would indeed be very surprising that it was not disclosed before, particularly since there has arguably been a long-felt need in the art for a straightforward and convenient method for assaying the downstream enzymes of the peptidoglycan biosynthetic pathway, particularly a method that can be used with membrane-bound enzymes (as demonstrated in the example of the present

application) and also one that can be adapted for high throughput screening of compounds.

Notwithstanding that the presently claimed invention is nonobvious in view of the above arguments, Applicants present the following additional arguments in support of nonobviousness.

In arguendo, even if the prior art could be considered to provide a suggestion of the presently claimed invention (which it cannot), at the very most this could only possibly be considered to make it obvious to try to obtain the present invention, rather than making the present invention obvious to do.

It is well-established that the standard of nonobviousness under 35 U.S.C §103 is *not obvious to try, but obvious to do*. (See In re O'Farrell, 853 F.2d 894, 7USPQ2d 1673 (Fed. Cir. 1988).) As determined in O'Farrell, an invention that is obvious to try is nevertheless nonobvious when the prior art makes it obvious to explore a new technology or general approach, for example SPA, that seemed to be a promising field of experimentation, but where the prior art gives only general guidance as to the particular form of the claimed invention or how to achieve it. The courts have also rejected an "obvious to experiment" approach; selective hindsight is no more applicable to the design of experiments than it is to the combination of

prior art teachings. (See In re Dow Chem. Co. 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).)

In view of Applicants' arguments in the previous sections, the cited prior art does not make the presently claimed invention obvious to do. A further indication that the prior art fails to make the presently claimed invention obvious to do is that the subject matter of the primary reference, Elhammer, and that of the present invention are only remotely related. Specifically, in contrast to the presently claimed invention, Elhammer does not relate at all to enzymes, such as bacterial GlcNAc-transferase, required for peptidoglycan synthesis. Furthermore, the peptidoglycan synthetic pathway is found only in bacterial (prokaryotic) systems. Elhammer, however, relates to the eukaryotic enzyme GalNAc-transferase, which catalyzes the transfer of N-acetylgalactosamine to serine and threonine residues of polypeptides to form glycosylated proteins. (See Elhammer, page 2, line 6 - page 3, line 8.)

Furthermore, Elhammer actually teaches away from the use of SPA when a membrane-bound enzyme is involved in the catalytic process or pathway for which an assay is desired. (See Elhammer, page 3, lines 9-15.) This makes embodiments of the present invention which utilize membrane-bound transferase *not even* obvious-to-try.

It must also be appreciated that Elhammer provides an assay for the activity of a single eukaryotic enzyme involved in a one-step process, the *O*-glycosylation of polypeptides. This enzyme is not part of the complex, multistep prokaryotic peptidoglycan synthesis pathway of the instant invention. Thus, Elhammer only teaches and provides motivation for a reductionist approach, focused on identifying inhibitors of the single enzyme. In contrast, the presently claimed invention employs a multi-enzyme, system-based approach by which compounds which inhibit any single activity, or even multiple catalytic activities, of the subject part of the peptidoglycan synthesis pathway can be identified. The system-based approach of the present invention provides the following advantages not provided by or suggested by the prior art.

First, when, as for the case of the present invention, the goal is to obtain inhibitors of the production of a product formed by a synthetic pathway, it is far more efficient to simultaneously screen for inhibitors of multiple parts of the pathway rather than to screen for inhibitors of each enzymatic activity separately. There is no suggestion of this advantage whatsoever in any of the cited prior art references.

Second, by measuring the success of inhibition of production of the end product, where it is the production of the end-product that is important (in this case, necessary for

growth and survival of bacterial pathogens), the assay of the present invention, in contrast to single-enzyme types of assays, assures that the effect of an inhibitor identified by the assay on an enzyme within the pathway segment is not countered by regulation of another enzyme, so that production of the end-product is unaffected.

Third, by employing an entire segment of the peptidoglycan synthetic pathway, the presently claimed assay provides for the possibility of identifying indirect inhibitors of enzymes within the subject pathway segment, i.e., inhibitors that do not directly inhibit a subject enzyme but that interfere with, for example, the allosteric regulation of the enzyme by another enzyme or by the upstream or downstream product of another enzyme. These sorts of inhibitors are not identifiable by a single-enzyme type of assay.

In view alone of the *per se* advantages described above, which are in no way suggested by or appreciated in the cited prior art, the presently claimed invention is nonobvious.

For all of the reasons set forth above, the present invention is nonobvious over the cited prior art. In summary, the Examiner has read far more guidance into the prior art than is warranted, and this is borne out by the facts, for example, that 1) the assay described in the primary reference and that instantly claimed cannot be said to be that similar and 2)

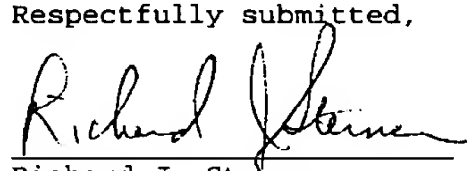
despite the guidance alleged by the Examiner, no one in the ten years prior to Applicants' filing of the application is on record as having thought of developing the assay claimed in the instant application. In light of the actual state of the prior art at the time of filing, which included disclosure actually teaching away from the instant invention, and in light of the advantages provided by the instant invention, said invention cannot be considered obvious in view of the cited prior art or in view of any other knowledge at the time.

In light of the amendments herein and the above arguments, the claims describe the invention with the definiteness required by statute, and the claimed subject matter is patentably distinct from the knowledge in the field at the time of filing. Reconsideration and allowance of pending claims 2-9 are respectfully requested. Should any other matters require attention prior to allowance, it is requested that the Examiner contact the undersigned.

The Assistant Commissioner is hereby authorized to charge any fees which may be due for any reason to Deposit Account No. 23-1703.

Dated: May 2, 2002

Respectfully submitted,



Richard J. Sterner
Reg. No. 35,372

Applicants' Agent
Customer Number 007470
(212) 819-8200

Agent's Direct Line:
(212) 819-8783

Enclosure

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

2. (twice amended) An assay for detecting peptidoglycan synthesis, which comprises the steps of:

(1) incubating a reaction mixture comprising in aqueous medium a uridine(5'-)diphosphate (UDP)-*N*-acetylmuramylpentapeptide, radiolabelled UDP-*N*-acetyl glucosamine, a source of divalent metal ions, a source of undecaprenyl phosphate, a source of peptidoglycan, a source of translocase enzyme, a source of transferase enzyme, a[s] source of transglycosylase enzyme, a source of transpeptidase enzyme and a source of lipid pyrophosphorylase enzyme, under conditions suitable for peptidoglycan synthesis;

(2) adding a divalent metal ion chelator compound to the reaction mixture of step (1) to terminate peptidoglycan synthesis;

(3) adding lectin-coated beads impregnated with a fluorescer to the reaction mixture of step (2), which beads bind any radiolabelled peptidoglycan synthesized in step (1); and

(4) measuring light energy emitted by the fluorescer, which energy is indicative of the presence of radiolabelled peptidoglycan synthesized in step (1).

Substrate Synthesis and Activity Assay for MurG

Hongbin Men, Peter Park, Min Ge, and Suzanne Walker*

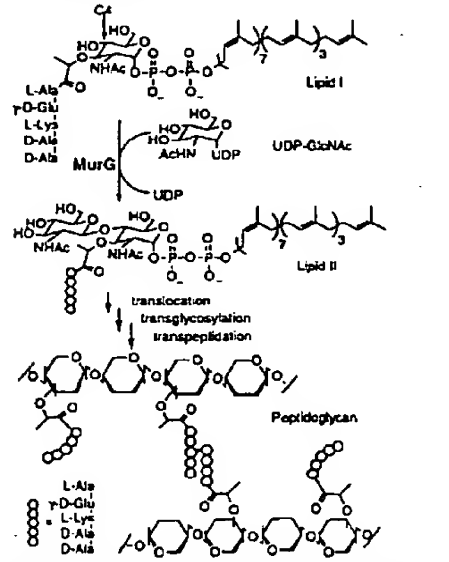
Department of Chemistry, Princeton University
Princeton, New Jersey 08544

Received December 15, 1997

Some of the best antibiotics function by interfering with the biosynthesis of the peptidoglycan polymer that surrounds bacterial cells. With the emergence of bacterial pathogens that are resistant to common antibiotics it has become imperative to learn more about the enzymes involved in peptidoglycan biosynthesis. Although remarkable progress has been made in characterizing some of the early enzymes in the biosynthetic pathway,[1] the downstream enzymes have proven exceedingly difficult to study. This is partly because the downstream enzymes are membrane-associated,[2] making them intrinsically hard to handle, and partly because substrates for many of the enzymes are not readily available.[3] These problems have impeded the development of activity assays suitable for detailed mechanistic investigations of the downstream enzymes.[4]

MurG, a cytoplasmic membrane-associated enzyme, catalyzes the transfer of UDP-*N*-acetylglucosamine (UDP-GlcNAc) to the C4 hydroxyl of an undecaprenyl pyrophosphate *N*-acetylmuramyl pentapeptide substrate (lipid I), assembling the disaccharide−pentapeptide building block (lipid II) which is incorporated into polymeric peptidoglycan (Scheme 1).[5] No mammalian homologues of this enzyme have been identified, and the muramyl pentapeptide substrate is unique to bacteria. These features suggest that it will be possible to design specific MurG inhibitors. However, despite decades of effort spent characterizing MurG activity, there is virtually no structural or mechanistic information on the enzyme.[6] Difficulties isolating lipid I have prevented the development of a simple, direct assay for MurG activity.[3,7] In this paper we report the synthesis of a substrate for MurG and demonstrate its use in an activity assay.

Scheme 1. The Reaction Catalyzed by MurG in the Context of Peptidoglycan Biosynthesis[a]



[a] The composition of the pentapeptide varies with microorganism, particularly at the third position (shown here as L-Lys).[13]

Our first synthetic target, 5a (Scheme 2), differs from lipid I in that the 55-carbon undecaprenol chain has been replaced by the 10-carbon chain of citronellol. A shorter lipid chain was chosen because long-chain lipids are difficult to handle; a lipid containing a saturated isoprenol unit was chosen because allylic pyrophosphates are unstable. Although MurG is a membrane-associated enzyme which recognizes a lipid-linked substrate, the chemistry takes place on the C4 hydroxyl of the lipid-linked substrate, which is far away from the lipid anchor; therefore, we anticipated that it would be possible to alter the lipid without destroying substrate recognition. To make 5a (Scheme 2), muramic acid derivative 1 (Sigma) was converted to the anomeric dibenzyl phosphate 2 in five steps and coupled to the protected pentapeptide 3.[8,9] Hydrogenolytic deprotection produced the anomeric phosphate 4, which was treated with diphenyl citronellol pyrophosphate.[10,11] The pyrophosphate exchange reaction took place readily in the presence of the unprotected sugar hydroxyls. Finally, the side chain protecting groups on the peptide were removed with TBAF, which also hydrolyzed the C-terminal methyl ester to give the desired product 5a. It should be noted that 5a is both acid- and base-sensitive. The synthesis minimizes exposure to acid and base, while providing for a convergent approach that allows independent modification of all three building blocks: the peptide, the carbohydrate, and the lipid. Thus,

(1) (a) Fan, C.; Moews, P. C.; Walsh, C. T.; Knox, J. R. *Science* **1994**, *266*, 439. (b) Benson, T. E.; Filman, D. J.; Walsh, C. T.; Hogle, J. M. *Nat. Struct. Biol.* **1995**, *2*, 644. (c) Jin, H. Y.; Emanuele, J. J.; Fairman, R.; Robertson, J. G.; Hall, M. E.; Ho, H. T.; Falk, P.; Villafranca, J. J. *Biochemistry* **1996**, *35*, 1423. (d) Skarzynski, T.; Mistry, A.; Wonacott, A.; Hutchinson, S. E.; Kelly, V. A.; Duncan, K. *Structure* **1996**, *4*, 1465. (e) Schonbrunn, E.; Sack, S.; Eschenburg, S.; Perrakis, A.; Krekel, F.; Amrhein, N.; Mandelkow, E. *Structure* **1996**, *4*, 1065. (f) Benson, T. E.; Walsh, C. T.; Hogle, J. M. *Biochemistry* **1997**, *36*, 806.

(2) (a) Gittins, J. R.; Phoenix, D. A.; Pratt, J. M. *FEMS Microbiol. Rev.* **1994**, *13*, 1. (b) Bupp, K.; van Heijenoort, J. *J. Bacteriol.* **1993**, *175*, 1841.

(3) (a) Pless, D. D.; Neuhaus, F. C. *J. Biol. Chem.* **1973**, *248*, 1568. (b) van Heijenoort, Y.; Gomez, M.; Derrien, M.; Ayala, J.; van Heijenoort, J. *J. Bacteriol.* **1992**, *174*, 3549.

(4) For a fluorescent assay to monitor MraY activity, see: Brandish, P. E.; Burnham, M. K.; Lonsdale, J. T.; Southgate, R.; Inukai, M.; Bugg, T. D. H. *J. Biol. Chem.* **1996**, *271*, 7609.

(5) (a) Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, 199. (b) Mengin-Lecreulx, D.; Flouret, B.; van Heijenoort, J. *J. Bacteriol.* **1982**, *151*, 1109.

(6) (a) Anderson, J. S.; Matsuhashi, M.; Haskin, M. A.; Strominger, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 881. (b) Anderson, J. S.; Matsuhashi, M.; Haskin, M. A.; Strominger, J. L. *J. Biol. Chem.* **1967**, *242*, 3180. (c) Taku, A.; Fan, D. P. *J. Biol. Chem.* **1976**, *251*, 6154. (d) Mengin-Lecreulx, D.; Texier, L.; van Heijenoort, J. *Nucleic Acid Res.* **1990**, *18*, 2810. (e) Ikeda, M.; Wachi, M.; Jung, H. K.; Ishino, F.; Matsuhashi, M. *Nucleic Acid Res.* **1990**, *18*, 4014. (f) Mengin-Lecreulx, D.; Texier, L.; Rousseau, M.; van Heijenoort, J. *J. Bacteriol.* **1991**, *173*, 4625. (g) Miyao, A.; Yoshimura, A.; Sato, T.; Yamamoto, T.; Theeragool, T.; Kobayashi, Y. *Gene* **1992**, *118*, 147. (h) Ikeda, M.; Wachi, M.; Matsuhashi, M. *J. Gen. Appl. Microbiol.* **1992**, *38*, 53.(7) Although lipid I can be isolated with difficulty,[6b] MurG activity is typically assessed using crude membrane preparations in which the lipid I substrate is generated in situ, and then the incorporation of radiolabel from radiolabeled UDP-GlcNAc into lipid-linked products is monitored.[2a,6b] This type of assay is adequate for evaluating inhibitors but is not suitable for detailed mechanistic or structural studies.

(8) Chen, J.; Dorman, G.; Prestwich, G. *J. Org. Chem.* **1996**, *61*, 393.

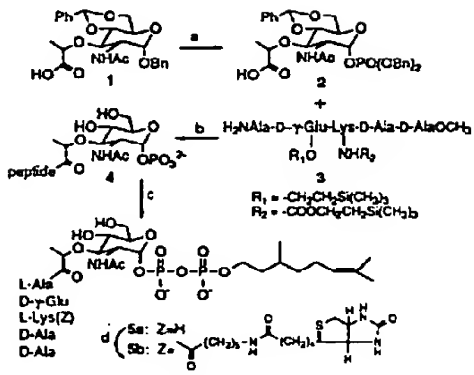
(9) The protected pentapeptide was synthesized on a D-Ala-FMOC Sasrin resin (Bachem Biosciences) in 11 steps in an overall yield of 15%. Experimental details are provided in Supporting Information.

(10) Diphenyl citronellol pyrophosphate was generated in situ by treating citronellol phosphate with diphenyl chlorophosphate (Supporting Information). See also: Warren, C. D.; Jeanloz, R. W. *Methods Enzymol.* **1978**, *50*, 122.

(11) For other methods to form glycosyl pyrophosphates, see: (a) Imperiali, B.; Zimmerman, J. W. *Tetrahedron Lett.* **1990**, *31*, 6485. (b) Wittmann, V.; Wong, C.-H. *J. Org. Chem.* **1997**, *62*, 2144.

S0002-7863(97)04221-2 CCC: $15.00
Published on Web 02/28/1998

Scheme 2[a]



[a] (a) (1) 2 equiv of CCl_3CH_2OH, 1.5 equiv of DCC, 0.1 equiv of DMAP, THF, room temperature, 4 h, 80%; (2) H_2/Pd, EtOAc, room temperature, 0.5 h; 10 equiv of $PhCH(OCH_3)_2$, 0.1 equiv of TsOH, DMF, room temperature, 10 h, 81%, 2 steps; (3) 2 equiv of $^iPr_2NP(OBn)_2$, 4 equiv of ^{1}H-tetrazole, CH_2Cl_2, −20 °C → 0 °C, 0.5 h, then 5 equiv of mCPBA, −40 °C → 25 °C, 2 h, 75%; (4) Zn dust, 90% AcOH/H_2O, room temperature, 1 h, 91%. (b) (1) 2 equiv of HOBt, 2 equiv of Py-Bop, DIEA, DMF, 0 °C, 0.5 h, 92%; (2) H_2/Pd, CH_3OH, room temperature, 0.5 h, then DIEA. (c) (1) 2 equiv of (*R*)-(+)-β-citronellol-$OPO_3PO(OPh)_2$, py, DMF, room temperature, 48 h, 30%, 2 steps; (2) 20 equiv of TBAF, DMF, room temperature 24 h, 57%. (d) 2 equiv of 6-[(biotinoyl)amino]hexanoic acid succinimide ester, 5 equiv of $NaHCO_3$, H_2O/Dioxane, room temperature 2 h, 76%.

using the same general scheme we should be able to make a variety of compounds to define the requirements for substrate binding.

Initial attempts to use substrate **5a** in MurG activity assay revealed problems in separating radiolabeled product from excess labeled UDP-GlcNAc. The evidence suggests, however, that MurG is relatively insensitive to the identity of the third amino acid in the peptide chain.[12,13] Therefore, we attached biotin (Scheme 2)[14] to the ε amino group of the lysine residue so that radiolabeled product can be readily separated from other radioactive components in the reaction mixture using an avidin-derivatized resin (Tetralin Tetrameric Avidin Resin, Promega). The ability of MurG to recognize the biotin-labeled substrate **5b** was evaluated by counting the radioactivity that binds to the resin after incubation of various crude membrane preparations with **5b** and [^{14}C]UDP-GlcNAc.[15] Reaction is rapid and efficient with a bacterial culture that overexpresses MurG but barely detectable with a culture expressing only endogenous levels of MurG (Figure 1; cf. curves A and E).[16] Heat treating the overexpressing cell lysate prior to adding it to the substrates prevents the reaction (Figure 1; cf. A and D). Hence, the reaction depends on the presence of active MurG. Furthermore, both the initial reaction rate and conversion to product increase with the concentration of **5b** (Figure 1; cf. A, B, and C).

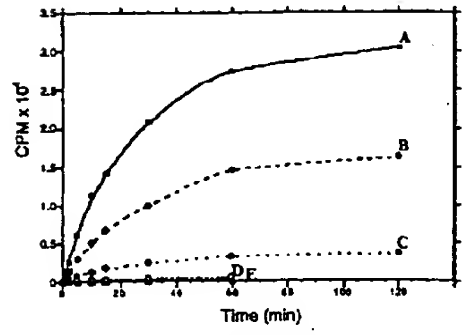


Figure 1. GlcNAc transfer as a function of [**5b**] and [active MurG]. All reactions were run in 100 mM Tris-HCl, pH 7.6, 1 mM $MgCl_2$, with 0.5−1.0 μg total protein and 9.4 μM [^{14}C]UDP-GlcNAc (265 mCi/mmol). Reactions for curves A, B, C, and D were carried out using a cell lysate from a transformed BL21(DE3)pLysS strain that overexpresses MurG: (A, ■) 7.1 μM **5b**; (B, ◆) 3.5 μM **5b**; (C, ●) 0.71 μM **5b**; (D, ○) 7.1 μM **5b** + heat-treated cell lysate (65 °C, 5 min). Reactions for curve E were carried out using a BL21(DE3)pLysS cell lysate expressing only endogenous levels of MurG: (E, △) 7.1 μM **5b**.

In conclusion, the synthetic substrate functions efficiently in a direct assay for MurG activity despite having a different, and dramatically shorter, lipid chain. It should be possible to use this synthetic substrate to evaluate enzyme activity in overexpressing cell lysates following structural modifications to the *murG* gene that produce amino acid truncations, additions, deletions, or mutations. The synthetic substrate can also be used to assay for enzyme activity during purification, as well as for detailed mechanistic studies on wholly or partially purified enzyme. These types of experiments will lay the foundation for high-resolution structural analysis of MurG. In addition, by evaluating the ability of other synthetic substrates to compete with **5b** for [^{14}C]UDP-GlcNAc, it should be possible to identify simpler acceptors for use in direct screens for MurG inhibition.

Acknowledgment. This work was supported by Interneuron Pharmaceuticals. We thank Transcell Technologies for generously providing a plasmid containing *murG*.

Supporting Information Available: Experimental procedures and spectral data for the synthesis of the protected pentapeptide and diphenyl citronellol pyrophosphate; spectral data for compounds **4**, **5a**, and **5b** (18 pages). See any current masthead page for ordering information and Web access instructions.

JA974221P

(12) *E. coli* strains (e.g., BL21) make a muramyl pentapeptide substrate with *meso*-diaminopimelic acid rather than L-lysine, but *E. coli* MurG accepts the lysine analogues.[6a,13] Fluorescently labeled analogs are also accepted by some strains: Weppner, W. A.; Neuhaus, F. C. *J. Biol. Chem.* **1978**, *253*, 472.

(13) White, D. *Physiology and Biochemistry of Prokaryotes*; Oxford Univ. Press: New York, 1995; pp 212-223.

(14) 6-[(biotinoyl)amino]hexanoic acid succinimide ester was purchased from Molecular Probes, Inc.

(15) Baker, C. A.; Poorman, R. A.; Kezdy, F. J.; Staples, D. J.; Smith, C. W.; Elhammer, A. P. *Anal. Biochem.* **1996**, *239*, 20.

(16) The *murG* gene was subcloned from a pET15b construct provided by Transcell Technologies, Inc. into a pET3a plasmid. MurG was overexpressed in the IPTG-inducible BL21(DE3)pLysS strain (Novagen). See: Studier, F. W.; Rosenberg, A. H.; Dunn, J. J.; Dubendorff, J. W. *Methods Enzymol.* **1990**, *185*, 60.